Use of FISH technique in the diagnosis of chromosomal syndromes

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SUMMARY Major chromosome abnormalities are present in 0.65% of all neonates. Fluorescent in situ hybridization (FISH) is useful in diagnosing microdeletion syndromes that would otherwise be difficult to diagnose using standard cytogenetics. In this study, we used FISH analysis in the laboratory diagnosis of 4 patients with Prader–Willi Syndrome [del(15)(q11.2q12)], 4 patients with DiGeorge syndrome [del(22)(q11.2q11.23)] and 4 patients with Williams syndrome [del(7)(q11.23q11.23)]. High-resolution chromosome analysis in all these patients was either normal or inconclusive but all the syndromes were confirmed using FISH. We recommend cytogenetic analysis should always be supplemented with FISH to diagnose all cases suspected of a microdeletion syndrome.

Introduction

It is well documented that 0.65% of neonates (1 in 156 live births) exhibit a major chromosomal abnormality [*1*]. These abnormalities fall into three main categories:

- numerical abnormalities resulting in aneuploidy;
- structurally balanced or unbalanced chromosome rearrangements;
- chromosomal microdeletions (CMD).

Although routine chromosome analysis is very precise in diagnosing both numerical and structural chromosomal abnormalities, it is less sensitive in the diagnosis of patients with CMD. Patients with CMD exhibit complex phenotypic abnormalities, typically described as contiguous gene-deletion syndrome [*2*]. High-resolution chromosome analysis in these patients generally does not demonstrate any visible abnormalities. However, in some of these patients, deletions of specific regions of a chromosome have been demonstrated.

Some common CMD syndromes include Prader–Willi and Angelman syndromes [del(15)(q11.2)], DiGeorge syndrome [del(22)(q11.2)], Miller–Dieker syndrome [del(17)(p13)] and Williams syndrome [del(7)(q11.23)]. Current laboratory diagnosis of CMD syndromes has been dramatically improved by the application of the fluorescent in situ hybridization technique (FISH) [*3,4*], a technique which makes use of the basic principles of molecular biology. A variety of FISH probes are available commercially, and can be used

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Received: 10/09/98; accepted: 02/09/99

with great confidence to diagnose both numerical and structural chromosomal abnormalities, as well as for single genes or gene clusters. FISH is used very effectively in diagnosing CMD syndromes that are otherwise difficult to diagnose using standard cytogenetics.

Here we present our data on the use of FISH in diagnosing three common CMD syndromes: Prader–Willi and Angelman syndromes (PWS/AS), DiGeorge syndrome (DGS) and Williams syndrome (WS).

Patients and methods

A total of 12 patients were referred from our genetics clinic after clinical evaluation by a staff clinical geneticist in order to rule out the possibility of DiGeorge syndrome (4 patients) (Table 1), Prader–Willi/Angelman syndrome (4 patients) (Table 2) and Williams syndrome (4 patients) (Table 3). All the patients had typical clinical features (Tables 1, 2 and 3). All patients were analysed by both high-resolution chromosome banding and FISH, using commercially available DNA probes. Metaphase chromosomes were stained with trypsin-Giemsa, with 20 metaphases screened and 2–5 metaphases karyotyped.

FISH analysis was carried out using appropriate DNA probes, either with biotin or digoxigenin-labelled DNA kits (Oncor Incorporated, Gaithesburg, Maryland, United States of America). For the diagnosis of DGS the D22S75 DiGeorge chromosome region probe with chromosome 15 control probe (Cat.#5152, Oncor Incorporated, United States of America) was used. Williams syndrome was confirmed by Elastin Williams syndrome chromosome region (WSCR) probe, premixed with D7S427 chromosome 7 control probe (Cat.#P5155,

Table 1 **Clinical features and laboratory findings in four patients with DiGeorge syndrome (DGS)**

Manifestation	Patients number (age) DGS1 1 month	DGS2 1 year	DGS3 7 years	DGS4 1 year
Congenital heart defect	+	+	–	+
Facial dysmorphology	+	+	+	+
Learning disability	NA	+	+	+
Mental retardation	NA	+	+	+
Thymic aplasia /hypoplasia	+	+	–	+
Hypocalcaemia	+	+	–	+
Growth retardation	+	+	+	+
Cytogenetic analysis	N	N	N	N
FISH del(22)(q11.2)	+	+	+	+

NA = not applicable, N = normal

Table 2 **Clinical features and laboratory findings in four patients with Prader–Willi syndrome (PWS)**

Manifestation	Patient number (age) PWS1 7 years	PWS2 1 year	PWS3 8 years	PWS4 8 years
Obesity	+	+	+	+
Short stature	+	+	+	+
Mental retardation /developmental delay	+	+	+	+
Hypogonadism	+	+	+	+
Typical faces	+	+	+	+
Small hands/foot	+	+	+	+
Hypotonia	+	+	+	+
Cytogenetic analysis del(15)(q11.2q12)	?+	?+	?+	?+
FISH	+	+	+	+

Table 3 **Clinical features and laboratory findings in four patients with Williams syndrome (WS)**

Manifestation	Patients number (age)			
	WS1 5 months	WS2 4 years	WS3 13 years	WS4 2.5 years
Periorbital fullness	+	+	+	+
Broad nasal tip	+	+	?	+
Anteverted nare	+	?	–	–
Sagging cheeks	+	+	+	–
Full lower lip	+	+	+	+
Open mouth	+	+	+	+
Developmental delay	+	+	+	+
Mental retardation	+	+	+	+
Dental anomalies	–	+	+	+
Cardiac anomalies	+	+	+	+
Cytogenetic analysis	N	N	N	N
FISH del(7)(q11.23)	+	+	+	+

N = normal

Oncor Incorporated). For the detection of PWS/AS cases, the SNRPN Prader–Willi/Angelman chromosome region probe with promyelocytic leukaemia protein chromosome 15 control probe (Cat.#P5152, Oncor Incorporated) was used. The FISH method used for probe hybridization was that recommended by the manufacturer. Metaphase cells were prepared from short-term blood cultures using standard cytogenetic procedures.

Following the harvest, these metaphase cells were fixed in 3:1 methanol:acetic acid and dropped onto glass slides. The slides were allowed to air dry for several hours prior to use. The FISH procedure was carried out in accordance with the procedure for the Oncor Incorporated hapten-labelled unique sequence probes [5]. In short, slides were first incubated for 30 minutes in 2 × sodium chloride/sodium citrate (SSC) solution followed by dehydration in 70%, 80% and 95% ethanol. Slides were denatured for 2 minutes in a 70% formamide/2 × SSC pH 7.0 solution at 72 °C, followed by dehydration in 70%, 80% and 95% ethanol at –20 °C. A 10 mL aliquot of appropriate Oncor microdeletion probe mixture, which comes premixed in 50% formamide/2 × SSC, was placed on the slide and covered with a 22 × 22 mm glass cover slip sealed with rubber cement. The slide was placed in a humidity chamber in an incubator at 37 °C. Single-strand probe and patient DNA were allowed to hybridize overnight for a minimum of 18 hours. The post-hybridization washing was done at 72 °C for 5 minutes, in a 2 × SCC solution, followed by immersion in Oncor's 1 × phosphate-buffered detergent (PBD). The microdeletion probes came labelled with digoxigenin and were detected by application of 60 µL rhodamine-labelled anti-digoxigenin in (Oncor Incorporated), followed by incubation for 5 minutes at 37 °C. The slides were washed once again in 1 × PBD, then counterstained with diaminophenyl indol and a cover slip was placed on top.

A minimum of 10–20 metaphases per patient were scored for the presence or absence of the critical region and control signals on both homologues for each of the probes. The hybridized-labelled DNA sequences were visualized on chromosomes and scored on a Zeiss Axiophot fluorescence microscope, equipped with 100 watt high-pressure mercury lamp. The FISH images were captured either on a 35 mm Kodak Ektachrome colour slide film (HC100) at ASA 400, or on PowerGene Karyotyping (Perceptive Scientific Instruments Incorporated, Texas, United States of America).

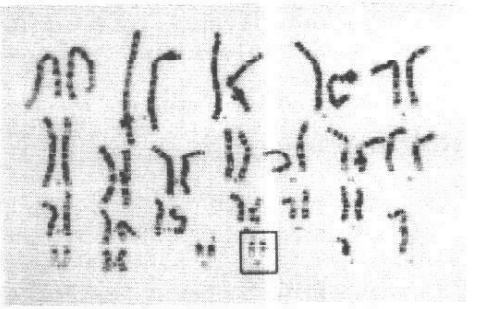

Figure 1a **A representative karyotype (46,XY) of a patient with DiGeorge syndrome; chromosome 22 pair is shown in the box.**

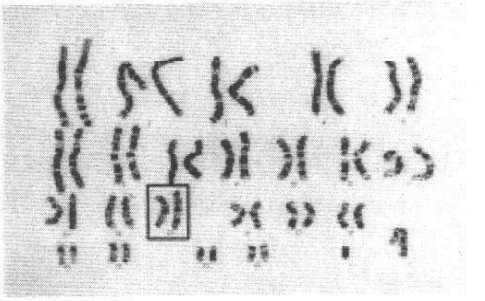

Figure 2a **A representative karyotype 46,XY,?del(15)(q11.2q12) of a patient with Prader–Willi syndrome (PWS); the deleted chromosome 15 is on the left in the box.**

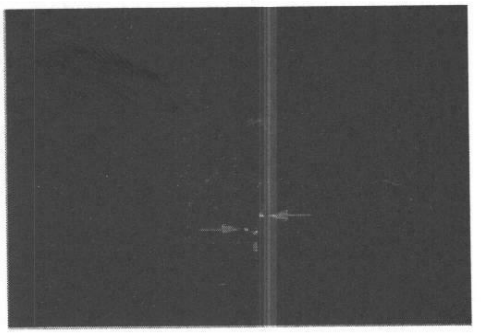

Figure 1b **FISH analysis showing the deletion of the DGCR [22(q11.2)] region. The small arrow points to the DGCR region deleted on one homologue of chromosome 22. The large arrows show the internal control probe for chromosome 22.**

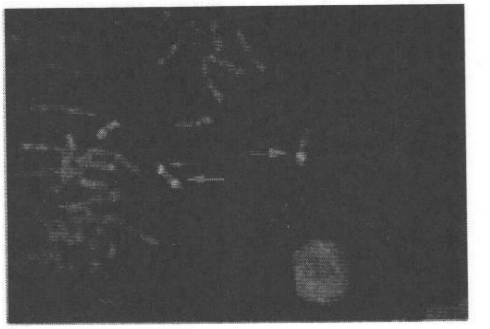

Figure 2b **FISH analysis showing the deletion of the PWS region on one homologue of chromosome 15 (small arrow). The large arrows show the internal control probe for chromosome 15.**

Results

The clinical and laboratory findings for DGS patients are summarized in Table 1. Of the 4 patients with DGS, chromosome results were normal in 3, while in 1 DGS case, a deletion in chromosome 22 at band q11.2 was suspected. A representative karyotype, with normal chromosomes and FISH image showing hemizygosity at the DG critical region (DGCR) locus are shown in Figure 1. All 4 DGS patients had a deletion of the DGCR on chromosome 22. In all 4 cases of PWS (Table 2), chromosome findings revealed a possible interstitial deletion in one chromosome 15 homologue at bands

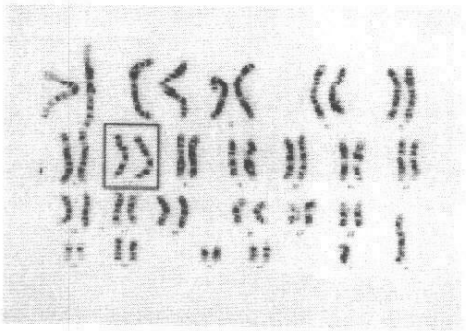

Figure 3a **A representative karyotype 46,XY of a patient with Williams syndrome; chromosome 7 pair is shown in the box.**

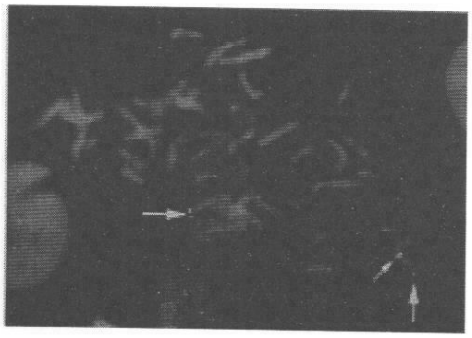

Figure 3b **FISH analysis showing the deletion of the elastin gene on chromosome 7 (small arrow). The large arrows point to the internal control probe for chromosome 7.**

q11.2q12. FISH analysis confirmed the deletion in all the PWS cases. A representative karyotype and FISH image with the deletion of PWS/AS critical region are shown in Figure 2. Of the 4 patients with Williams syndrome, 2 had a normal karyotype. The other 2 showed a possible interstitial deletion on one chromosome 7 homologue at band 7q11.23 (Table 3). A representative karyotype and FISH results are shown in Figure 3. All cases showed hemizy- gosity for the elastin gene by FISH analysis.

Discussion

Microdeletion of chromosome region 22q11.2 is associated with DGS, velocardiofacial syndrome, conotruncal facial abnormality and other types of cardiac defects [*6,7*]. DGS is characterized by a more severe clinical phenotype. Approximately 90%–96% of DGS patients show a microdeletion of the 22q11.2 region [*7*]. Establishing a diagnosis by high-resolution chromosome analysis can be very difficult. However, the usefulness of FISH in detecting the 22q11.2 deletion has recently been well documented [*9*]. All patients with DGS in our study had normal karyotypes by high-resolution chromosome analysis, except in one case where a deletion in the 22q11.2 region was suspected. However, using FISH technique with DGCR probe, all 4 patients were confirmed as having a 22q11.2 deletion, thus establishing DGS in these patients (100% concordance).

PWS is a complex CMD syndrome with an incidence of 1 in 30 000 [*10*]. In 70% of PWS patients, an interstitial deletion of the (15)(q11q13) region derived from the paternal chromosome is a characteristic feature [*11,12*]. About 30% of PWS cases are due to maternal uniparental disomy [*13,14*]. FISH has recently been used successfully in detecting PWS patients with a deletion [*15,16*]. We studied 4 patients to rule out or confirm the possibility of PWS. All of these patients had a suspected interstitial deletion at the (15)(q11q13) region by high-resolution chromosome analysis. FISH analysis with PWS region probe confirmed the deletion in all cases.

The incidence of WS is estimated to be between 1 in 20 000 and 1 in 50 000 live

births [*17*]. The characteristic clinical features of the WS patient are periorbital fullness, congenital heart defect, developmental delay, mental retardation, learning disability and infantile hypercalcaemia [*17,18*]. Many WS patients are not diagnosed until they are old, when they show characteristic personality and facial changes [*19*]. Since the phenotype becomes variable with advancing age of WS patients, diagnosis becomes more challenging [*20*]. However, since complete deletion of one elastin allele on chromosome 7 at band 7q11.23 in WS patients has been demonstrated, diagnosis is now easier [*21*]. All classical cases of WS have recently been shown to have a deletion of elastin locus by FISH analysis [*20,22*]. While in our 4 WS patients, chromosome analysis was normal, a deletion of elastin locus was seen in all 4 patients by FISH analysis.

Since many of the CMD syndromes exhibit a broad range of phenotypes, clinical diagnosis can be difficult. Cytogenetic analysis alone can give ambiguous results. Application of the FISH technique in laboratory diagnosis of CMD syndromes has been shown to be extremely useful in many previous studies as well as in our study. Because some cases of CMD syndromes are due to mechanisms other than chromosome deletion, conventional cytogenetics should also be used routinely along with FISH. If a deletion in a CMD syndrome is found, parental chromosome studies are recommended in order to rule out any rearrangement involving the critical region. This information is crucial for assessing recurrence risk and for genetic counselling.

Acknowledgements

We wish to thank the technical staff of the Cytogenetics/Molecular Genetic Section for their efforts. We also thank Rita Johnston for her secretarial assistance.

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